EXAMINING THE FLEXIBILITY OF TIM BARREL PROTEINS BASED ON THEIR STRUCTURAL TOPOLOGY

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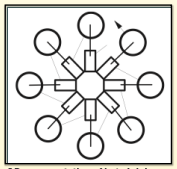
The eight-fold beta/alpha barrel-like fold, first seen in triose-phosphate isomerase (TIM), is a common and versatile fold for proteins. Proteins in this classification have diverse enzymatic functions spanning five of six Enzyme Commission classes[1].

Studies have characterized the sequence, structure, localized dynamics and electrostatics of TIM proteins, but the flexibility of the fold is understudied.

We report that **the eight core beta strands behave independently of the eight flanking alpha helices**. The strands and helices repeat in tandem at the sequence level, yet the strands form a core that moves minimally while the helices display greater relative mobility in all of the structures.



2D representation of beta/alpha barrel fold. The rectangles represent strands while the circles are the flanking helices. Reproduced with permission[2].

Normal Mode Analysis

The normal modes are eigenvectors of the matrix of the second derivatives of the potential for each atom in a given structure.
For the coarse-grained analysis, we used a form of Elastic Network Model defined by the C-alpha forcefield[3] in MMTK[4] to simplify computation. The all-atom analysis was performed using MMTK using the Amber99 force field[5]. The structure was minimized using 10 000 steps with the steepest descent algorithm, and subsequently conjugate gradient algorithm until convergence was reached. Then the matrix was diagonalised to retrieve the normal modes allowing only rigid body movement of the residues[6].

Atomic fluctuations

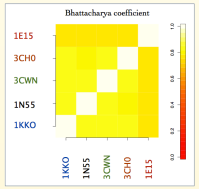
$$f(i) = \frac{1}{m_i} \sum_{x=0}^{3N-6} \frac{\mathbf{v}_{xi} \cdot \mathbf{v}_{xi}}{\lambda_x}$$

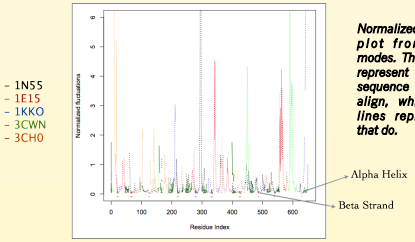
The fluctuations refer to the normalized squared fluctuations (or RMSF) given by the equation. Here, m refers to the mass of the atom, v_{xi} refers to the vector of the atom i in mode x and λ_x the eigenvalue of mode x. These values correspond to theoretical B-factors for all modes excluding the first six rigid-body modes.

To do the comparative analysis between multiple TIM barrels, structural alignments done using Mustang[7] were used.

We looked at the principle TIM beta/alpha barrel secondary structure elements of **five structures** from distinct families with **low sequence similarity** between 23 and 38.5 % and low structure similarity.

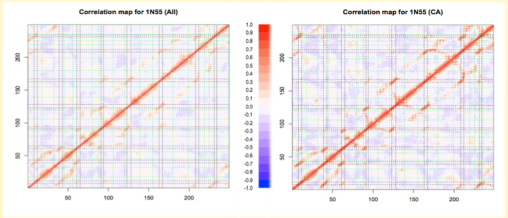
Despite the low sequence similarity, we found that **the normal modes at the points of structural similarity shows that there is a conservation of dynamics.** This is seen in the score given by the Bhattacharya coefficient[8].



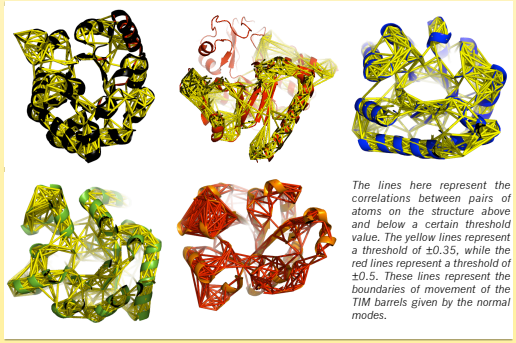


Normalized fluctuations plot from all-atom modes. The dotted lines represent parts of the sequence that does not align, while the solid lines represent parts that do.

We found that the atomic fluctuations of each of the beta-strands have **a high correlation with its immediate beta-strand neighbour in the core**. Compared to the beta strands, the alpha helices have less correlation with each other and almost none with the strands.

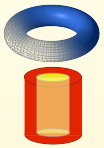


All-atom vs. coarse-grained correlation matrices. The matrix represents the correlation between the atomic fluctuations of pairs of atoms within a structure.

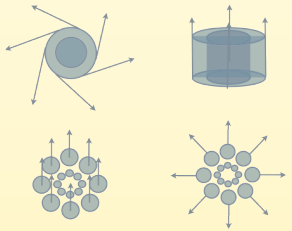


The lines here represent the correlations between pairs of atoms on the structure above and below a certain threshold value. The yellow lines represent a threshold of ±0.35, while the red lines represent a threshold of ±0.5. These lines represent the boundaries of movement of the TIM barrels given by the normal modes.

This core formed by the beta-strands is rigid in all the structures. This is despite the varying loop lengths between them and the presence of accessory secondary structures in some cases. This leads us to hypothesise that the movements of the TIM does not follow its **toroidal** structural description but rather as a **barrel within a barrel**.



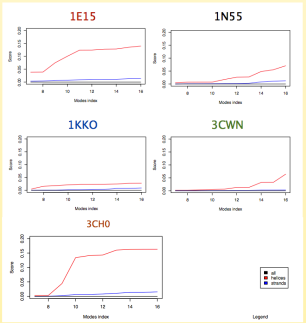
We also defined rotation and translation vectors to decompose the type of movements to which the normal modes of these TIMs correspond, based on the barrel-in-barrel description.



Vector diagrams describing:
Barrel rotation
Barrel translation
Individual helix/strand translation
Individual helix/strand sliding

We show an example of a result from the methods above in characterising the nature of TIM Barrel's flexibilty at the fold level.

The cumulative overlap from Barrel rotation showing the motions of the helical bundle and the beta-barrel core. While the helical bundle seems to have a greater preference for rotational motion in comparison with the beta barrel core, it varies quite greatly from structure to structure.



In conclusion, the normal modes of TIM are able to characterise its flexibility at the fold level, which gives us insight into its versatility. Our methods can pick out the commonalities between these proteins, such as the rigidity of the beta-barrel core, despite the low sequence and structure conservation.

References
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